Continuous-Wave Photoacoustic-Based Sensor for the Detection of Aqueous Glucose: Towards Non-invasive and Continuous Glycemia Sensing

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Abstract. Measurement of blood glucose levels (BGLs) is a basic procedure that diabetic patients need to perform several times a day. The conventional standard protocol for on-site measurement, despite several advantages such as portability, low cost, fast response time, and ease of operation, is based on the finger-prick technique to extract blood samples. This process is invasive and cannot provide continuous monitoring.

Towards the achievement of non-invasive and continuous BGL monitoring, we have developed two measurement methods based on the continuous-wave photoacoustic (CW-PA) protocol and we performed preliminary *in vitro* tests with aqueous solutions. The first method relies on the measurement of the frequency shift induced by the change in the composition of the propagation medium. This method is equivalent to an acoustic velocity measurement and provides high sensitivity but no selectivity to glucose compound. The second approach utilizes simultaneous optical excitation at two wavelengths for compound-selective measurements. After correcting the frequency shift mentioned previously, this protocol allows measurements equivalent to a differential absorption coefficient one at the two wavelengths used. It then combines the advantages of absorption spectroscopy without the limitation from scattering due to the use of acoustic detection. Furthermore, the combination of the two methods can be generalized to systems involving more than one changing parameter by using not only two optical wavelengths for the excitation sequence but also several pairs of wavelength sequentially.

These methods then represent an important step forward the non-invasive, selective, and continuous measurements of glucose compound concentrations from a complex mixture, typically blood.

Keywords: photoacoustic method, continuous blood glucose level.

1 Introduction

Diabetes mellitus, often referred to as diabetes, is a metabolic disorder characterized by hyperglycaemia (raised blood sugar levels) as result of less control of the

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blood glucose level (BGL). Despite symptoms being first described several hundred years ago, this illness still remains a serious issue for an estimated affected population exceeding three-hundred million worldwide in 2011 [1,2], which is further increasing yearly. Despite extensive investigations covering a large span of expertise, the exact cause of diabetes remains unknown, and a cure has not been discovered yet. However, repeated and long-lasting exposure of internal organs to abnormally elevated blood glucose levels (*i.e.* hyperglycaemia) results in multiple complications and premature mortality [3,4]. Tremendous efforts have been dedicated to the design of an efficient way to manually monitor and control the patients' BGL.

Maintaining the BGL within the range of variation expected from a healthy person is the basic way to prevent any impact of diabetes on patient health. Every time the BGL exceeds the normal limit value (several standards for diagnosis, always in the range of 100-200 mg/dL, have been published by the American Diabetes Association [5,6]), adequate actions, which depend on the type of diabetes, should be taken to restore its level into the admissible range. However, the efforts and actions taken to lower the BGL also expose the patients to hypoglycemia, which corresponds to a BGL lower than the limit value in the range of 70 mg/dL. Accurate, on-site and real time detection of BGL then represents the first essential step for adequate decision making. Several commercially available sensors based on blood sample analysis have been developed by numerous companies over the past decades. Exhibiting compact size, low cost, good accuracy, and fast response [7-9], they have rapidly become popular around the world and have provided huge benefits to the diabetic population. However, despite tremendous efforts to reduce the blood sample volume and the discomfort of finger-pricking, they are still invasive and cannot provide continuous monitoring, which is the corner-stone for optimal BGL control [10,11].

As one step towards this ultimate goal, minimally invasive (MI) techniques have been developed, where the sensor head reduced to its minimum size is inserted subcutaneously in direct contact with body fluids, while the signal processing and so on are performed outside the body. This approach consequently reduces the invasiveness and enables continuous monitoring over several consecutive days [12-15]. Furthermore, systems coupling a MI sensor to a insulin pump [16,17] have been developed for automatic delivery based on continuous measurements and a complex algorithm [18].

At the time of this writing, three products have received FDA approval for commercialization in the US. However, two main issues limit their application: (1) the frequent need for calibration and (2) the lack of accuracy in some particular cases, so that patients are additionally advised to perform regular tests before taking any potential life-threatening action. Implanting a device in the body, despite the reduced size and minimizing discomfort, also poses the problem of biofouling [15]. As a result, the commercially available products claimed to provide accurate readings for at least three days, although researchers are developing strategies for long-term readings for at least several months [20,21].

Though less advanced than the MI protocols, the non-invasive (NI) methods remain without question the preferred solution for sensing mechanism [22,23]. However, two main issues should be addressed with particular care: sensitivity and selectivity. Among the various alternative techniques that have been extensively studied over the last few decades [24-29], near-infrared (NIR) absorption spectroscopy has probably received the most interest because the method takes advantage of the finger-print-like absorption coefficient of each compound in this wavelength range. Furthermore, recent development of multivariate statistical algorithms applied to chemical problems (chemometric-based methods) have further extended the scope of the NIR approach by allowing efficient extraction of parameters of interest from complex systems involving simultaneous measurements at several wavelengths. However, this method's sensitivity to the scattering properties of tissues has prevented, at the time of this writing, any device to reach the commercialization stage. In contrast, photoacoustic-based (PA) protocols, which use optical excitation and mechanical detection, show great potential, offering high sensitivity and robustness against the scattering properties of tissue [28]. As a result, despite several on-going issues specific to the PA approach, we chose to investigate it and have developed two methods that focus on a very specific part of the detection scheme.

This chapter provides an overview of these methodologies, from the basic concept of PA technique to the *in vitro* characterization of aqueous solutions. It then describes in detail the combination of the two methods, which opens the door to multivariate measurements, similar to the popular NIR absorption spectroscopy methods (with the possibility of using chemometrics as well) mentioned previously but without the limitation due to scattering.

2 Continuous-Wave Photoacoustic (CW-PA) Procedure

Among the potential techniques, the PA techniques also fulfill all the requirements for non-invasive sensing of blood glucose levels. The concept can be briefly described as follows. An amplitude-modulated optical source operating at an adequate wavelength illuminates an absorbing medium, where optical energy absorption yields a temperature increase due to the non-radiative relaxation photothermal effect. This temperature increase results in volume expansion that will locally generate a pressure disturbance. This pressure perturbation can then propagate as a pressure/acoustic wave through the medium to a mechanical sensor, where detection of signal characteristics and its proper post-processing enables one to characterize the absorbing medium. This method has been claimed to be highly sensitive to glucose because this compound affects several parameters that strongly impact the process described above at several stages: i) the optical energy absorption, which involves the optical absorption coefficient, thermal expansion coefficient, heat capacity, and acoustic velocity; and ii) the acoustic wave propagation until the receiver by the means of the acoustic velocity [30].

Figure 1 (left) shows a schematic view of the minimal setup required to perform PA measurements. Light source locally illuminates the sample under investigation, and a transducer senses the mechanical wave. On one side, the use of optical fibers is very convenient since it allows flexibility (alignment between the light source and sample) without impacting the signal properties (low propagation losses over the wide optical range, robustness, wide range of diameter cores). On the other side, several ways of detecting the acoustic waves have been developed, but the use of transducers, despite requiring a direct mechanical contact with the sample, remains the standard technique.

In terms of optical wavelengths, the PA technique allows the use of the entire infrared spectrum (typically, from 0.8 to 1000 μm) to generate acoustic waves through the photothermal effect. However, the mid- and far-infrared (MIR from 2.5 to 25 μm, and FIR from 25 to 1000 μm, respectively) can excite the fundamental vibrations and associated rotational-vibrational structure, while the NIR region (from 0.8 to 2.5 μm) is associated with overtone or harmonic vibrations. The MIR and FIR wavelength ranges are then more suitable for finger-print-like absorption spectroscopy, which is critical when measuring a solution with several solutes. However, the absorption coefficient should also be considered from the point of view of the PA technique as well as the final application. Water, as the main constituent of human tissue, strongly absorbs light in the infrared region. However, a minimum absorption is required in order to efficiently generate acoustic waves within the tissue (for wavelengths higher than 1300 nm), while a strong absorption (typically, for wavelengths in the MIR or FIR range or above 2500 nm) strongly limits the depth penetration of optical light to superficial layers of the skin. We then chose to use the wavelength range from 1300 to 2500 nm, which is also referred to as the tissue optical or the therapeutic window [24,31].

As shown in Fig. 1, two protocols using different optical excitation patterns have been used extensively: the pulse setup (pulse of light, very low duty cycle, and frequency (or repetition rate) below 1 kHz), and the CW one (excitation with square-wave signal, duty cycle about 50%, frequency up to 1GHz). Both techniques can potentially provide high sensitivity, but one issue remains of particular importance in choosing the most appropriate excitation sequence to the target application: the dominant origin of noise affecting the measurement [32,33]. The pulse setup operates in the time domain with time gating to suppress the noise contribution. This technique is particularly suitable when dealing with systemic noise. On the other hand, the CW setup operates in the frequency domain (with the use of closed-loop and lock-in detection), which allows the use of filters to suppress the noise contribution. This technique is particularly suitable when dealing with random noise.

With *in vivo* environment, where the sample size and properties are difficult to control and stabilize along time, the noise source may more likely be assimilated to random, which points to CW as the preferable excitation sequence. Moreover, CW-PA exhibits other advantages in terms of the potential to facilitate miniaturization down to a portable size [34-36]. With the recent development of solid-state

laser diodes (LDs), compact high-power, high-resolution light sources covering this full range have become commercially available at reasonable cost and such light sources are particularly suitable components for CW-PA spectroscopy measurements. However, almost exclusively the pulse methodology has been cited in the literature dedicated to non-invasive glucose monitoring [27-29]. In fact, CW-PA methods were disregarded in a very early stage due to their dependence on cavity dimensions, which are parameters impossible to control precisely when it comes to dealing with real patients.

In this chapter, we describe, from the basic concept to the first *in vitro* results, two experimental protocols that allow CW-PA-based measurements whatever the cavity size and therefore solve the main issue usually associated with the CW-PA technique. Despite further optimization required to assess other characteristics such as detection limit and selectivity, these methods may represent a major breakthrough and initiate the future development of various types of sensors utilizing the CW-PA technology.

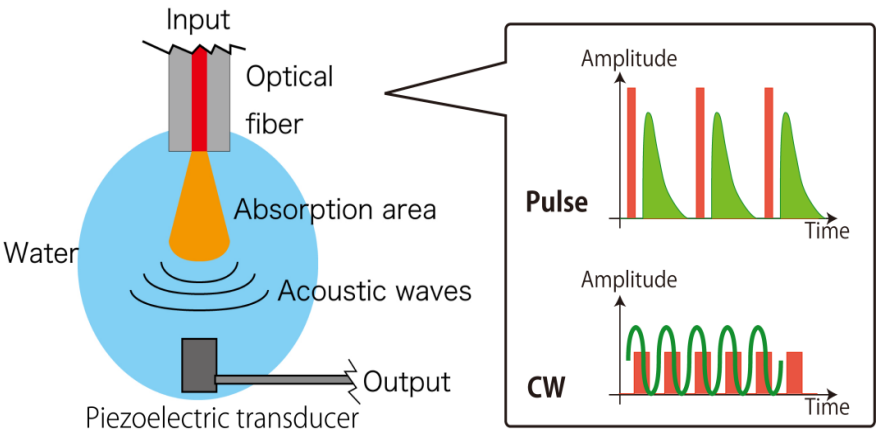

Fig. 1. Schematic view of the PA measurement cell (left), with the two optical excitation sequences used to generate the mechanical waves from photothermal effect (right)

2.1 Frequency Shift (FS) Protocol

The first protocol developed, called frequency shift (FS), relies on the measurement of the frequency shift at which acoustic resonance occurs when the glucose concentration of the sample solution is changed [37,38]. Figure 2 shows a schematic view of the experimental setup required to perform measurements based on the FS method.

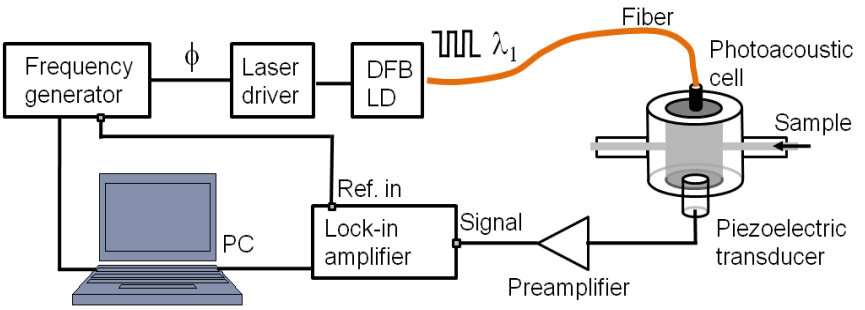

Fig. 2. Schematic view of the experimental setup used to perform FS-based measurements of various aqueous sample solutions

2.1.1 Concept

As stated above, the CW-PA technique generates standing acoustic waves within the cavity (volume with boundaries defined by strong acoustic impedance mismatch that consequently reflects acoustic energy). At certain frequencies, all the contributions will superimpose constructively and enhance the signal amplitude significantly. These frequencies depend on two factors: i) the boundary geometry, a geometrical factor that directly affects the acoustic wavelength, and ii) the mechanical properties of the sample liquid, all included within the acoustic velocity term. As a result, the resonant frequency can be described by the following equation:

$$f_{res} = v_{ac} / \lambda_{ac} \tag{1}$$

In some particular cases where the resonant cavity exhibits a simple geometry, analytical expressions have been derived to predict the resonant frequencies. With a one-dimensional cavity, the acoustic wavelength λ_{ac} of the m^{th} longitudinal mode can be defined as twice the cavity length divided by the integer m. With a cylindrical cavity, the analytical expression involves longitudinal as well as azymuthal and radial modes [39]. However, Eq. (1) still applies when all the geometrical factors within the λ_{ac} term are included.

When the glucose concentration of the sample solution is changed, the frequency at which the resonant occur shifts by a certain quantity Δf_{res}. However, from Eq. (1), as long as the geometry remains constant and the same mode is considered (peak in the closest vicinity of the previous one), the term λ_{ac} is constant, whereas adding glucose induces a change of the acoustic velocity. The shift of the frequency then comes exclusively from the acoustic velocity variation:

$$\Delta f_{res} / f_{res} = \Delta v_{ac} / v_{ac} \tag{2}$$

This protocol then enables one to measure the glucose concentration through its effect on the acoustic velocity whatever the cavity geometry, since Eq. (2) doesn't involve the acoustic wavelength anymore.

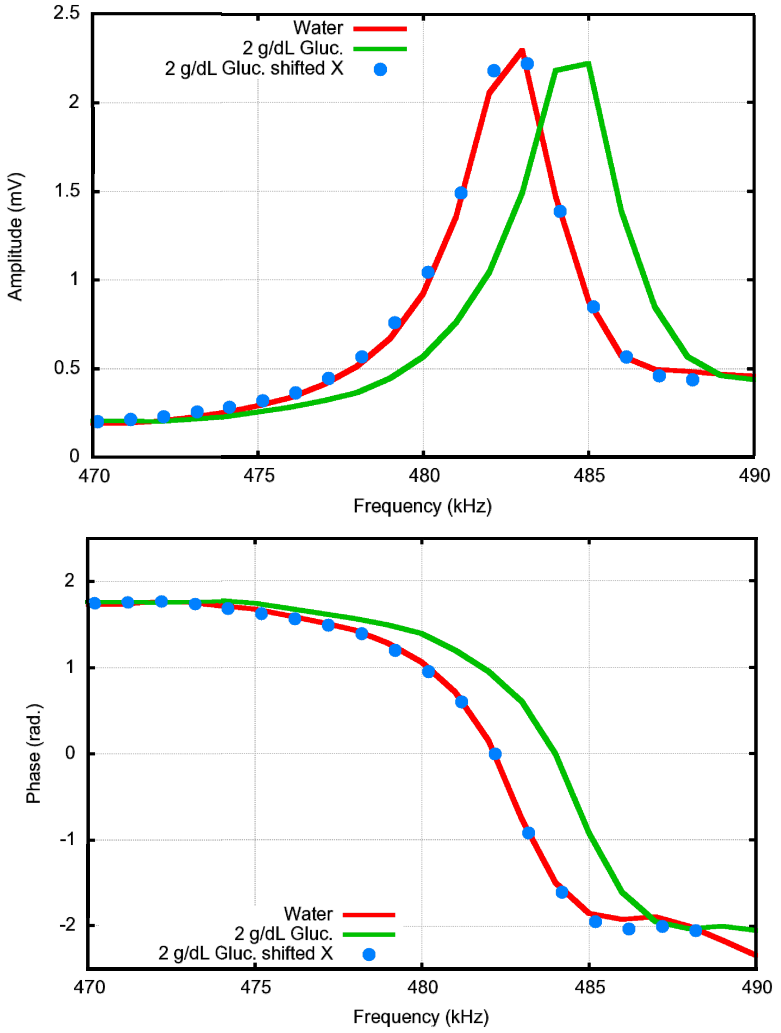

Fig. 3. Amplitude (top) and phase (bottom) raw results around one resonant peak at two glucose concentrations (0 and 2 g/dL, lines), and the 2 g/dL-response shifted to compensate for the effect of glucose concentration increase (dots)

2.1.2 Concept Proof of FS

The experimental setup shown in Fig. 2 allows the capture of both the amplitude and phase signals. Figure 3 shows raw experimental results around one resonant peak (arbitrarily chosen among several available resonant peaks within the full range spectrum (300-600 kHz)) when the glucose concentration is changed from 0 to 2 g/dL. These experiments were performed with the LD operating at 1382 nm. Despite the 1-kHz frequency step used to scan over a wide frequency range, the

frequency shift induced by the change in glucose concentration is obvious on both the amplitude and phase signals. It should also be noted that the same frequency shift on both the amplitude and phase enables us to compensate for the effect of glucose (dotted response in Fig. 3). However, the phase exhibits a linear tendency locally around the resonant frequency that allows fast and easy measurement of the shift, while the Gaussian-like shape of the amplitude requires a more complex algorithm. The FS protocol then preferably uses the phase to measure the shift of the frequency.

Figure 4 shows a set of results for 1610-nm excitation wavelength and glucose aqueous solutions with concentration levels up to 15 g/dL. Once more, a similar overall shift appears on both the amplitude and phase signals, combined with a peak maximum variation on the amplitude signal. Despite a difference between the 0 and 15 g/dL responses of about 20 %, *i.e.*, a level about 10 times higher than the frequency shift from the same glucose concentration increase, the huge background level as well as the absence of a clear tendency doesn't allow easy and precise measurements from the amplitude variations.

The proposed FS protocol then provides better sensitivity and linearity than amplitude-based measurements.

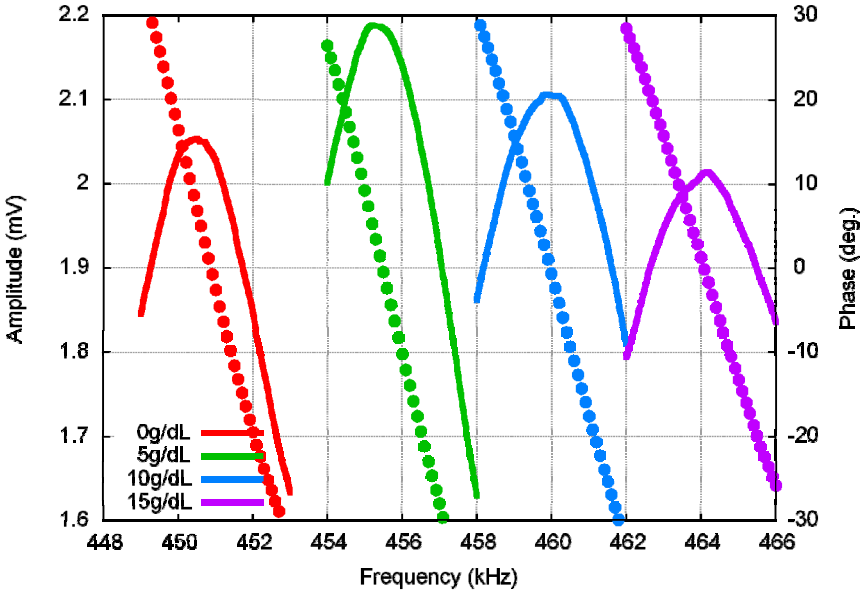

Fig. 4. Amplitude (lines) and phase (dots) signals of FS-based response performed with 1382-nm excitation wavelength and four glucose concentrations of aqueous solutions

2.1.3 Glucose Dependence at Various Conditions

The same experimental procedure was repeated several times with different cavity size (cylindrical shape with the length continuously varying from a few millimeters to few centimeters), optical excitation wavelength, and frequency (*i.e.*, different acoustic modes). The results shown in Fig. 5 reveal stable glucose concentration dependence with a linear response characterized by a 0.19 ±0.01 %/g/dL slope.

This result cannot be directly compared to results in the literature since this technique has not been used previously according to our knowledge. However, as mentioned previously, the proposed method is equivalent to a relative acoustic velocity measurement. Several papers deal with glucose concentration dependence of acoustic velocity, with results varying greatly between 0.15 [40], 0.20 [41], and 0.28 %/g/dL [28]. Despite the various methods (pulse photoacoustic, pulse-echo methods) and different accuracy, all the values are consistent with the FS response reported in this manuscript. The stable response despite changing the cavity geometry, the optical wavelength, and the mode considered also validates our first assumption that the FS method is equivalent to a relative acoustic velocity measurement.

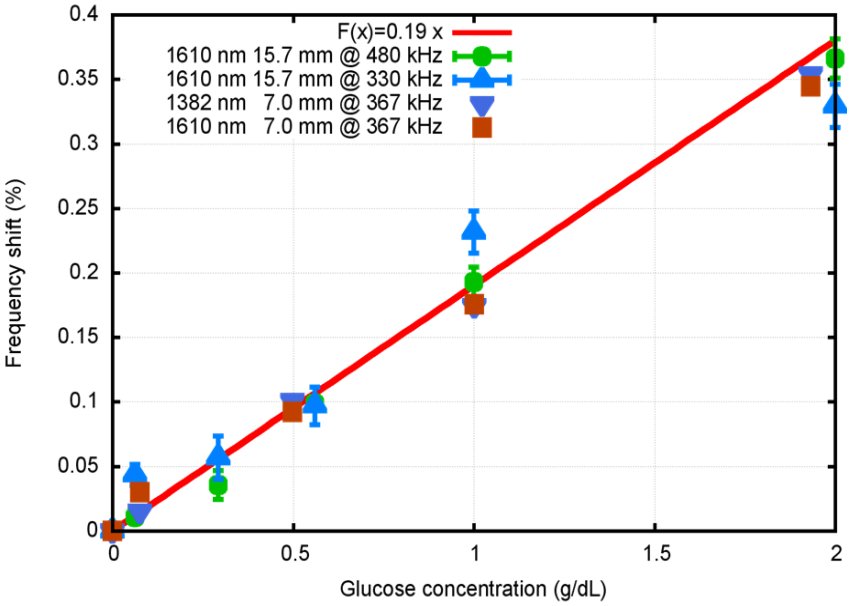

Fig. 5. Glucose concentration dependence of FS-based response at various conditions

This approach, by providing stability (versus optical wavelength, cavity size) and potential high sensitivity, then solves most of the issues usually associated with CW-PA technique. However, this approach also exhibits two main drawbacks: the impossibility to optimize the sensor response (a corollary of the previously mentioned stability) and the lack of selectivity to glucose compound in particular.

2.1.4 Issue of Selectivity to Glucose

The acoustic velocity depends on the glucose concentration, as well as other parameters such as temperature and other solutes' concentrations. Among the potential interfering parameters in clinical measurements, particularly concerns are related with the albumin and the temperature. We therefore further investigated the dependence of FS method to these two parameters. As an example, Fig. 6 shows the FS response with pure water at three different temperatures.

Despite changing exclusively the temperature, the effect on the sensor response is similar to the one induced by a change in the glucose concentration: a shift of the amplitude/phase signals and a variation of the maximum level on the amplitude.

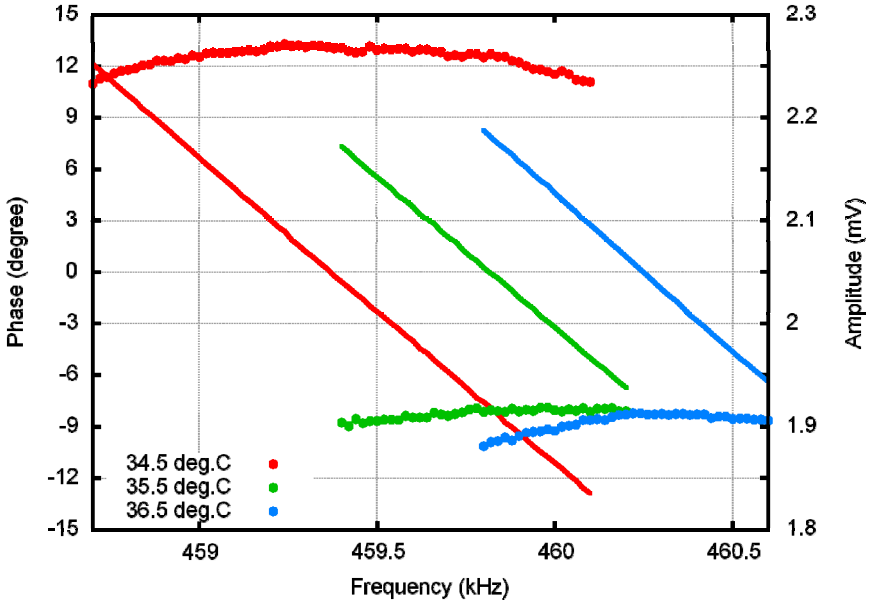

Fig. 6. FS response with pure water solution at three temperatures

As a consequence, the FS response exhibits sensitivity to temperature and albumin concentration with a slope of 0.16 %/ºC and 0.15 %/g/dL, respectively. However, when dealing with a sample where several parameters can vary simultaneously and independently (particularly the case with *in vivo* experiments), it is

impossible from the FS measurement, which is a scalar parameter, to separate the effect of glucose concentration change from temperature variations or albumin concentration changes. For example, a variation of 1ºC may be misinterpreted as an increase of glucose concentration by 0.84 g/dL from the FS measurement only. Therefore, the FS method is not sufficient to measure BGLs with satisfactory accuracy without the assumption that only one parameter is changing at a time.

2.2 Optical Power Balance Shift (OPBS) Protocol

The FS method relies on the measurement of the frequency shift of the phase signal. However, as shown in Figs. 4 and 6, the level of amplitude signals also varies in a irregular manner, depending on the glucose concentration. This non-trivial dependence results from the concomitant effects of several parameters (acoustic velocity, heat capacity, thermal expansion coefficient, optical absorption). The amplitude signal therefore contains valuable information about the sample solution characteristics that requires a specific measurement scheme. We then proposed the so-called optical power balance shift (OPBS) methodology in order to achieve a measurement that depends exclusively on the optical absorption coefficients.

2.2.1 Concept of Dual Differential Wavelength Excitation

From theoretical considerations, the pressure wave generated by illuminating the absorbing medium with an amplitude-modulated light beam depends on many parameters, such as the thermal expansion coefficient, acoustic velocity, heat capacity, optical absorption, and optical power [32,33]. Among these parameters, optical absorption is of particular interest because it provides a specific signature for every compound, and selection of the optical wavelength enables one to optimize the sensor response to a specific solute (the concept extensively used in NIR [42,43] and MIR spectroscopy [44,45] protocols). In the NIR region, water provides strong absorption that allows efficient generation of pressure waves by means of the photothermal effect. However, despite a concentration in the gram per deciliter range, consequently higher than the expected *in vivo* levels, the relative absorption of glucose and albumin compounds are several orders of magnitude lower than that of water. As a consequence, diluted compounds act as a perturbation to the huge background level provided by water solvent. Furthermore, glucose and albumin exhibit similar overall absorption spectra with slight differences at certain wavelengths (Fig. 7(a)). To overcome these two issues, we then used an excitation sequence with two optical beams at different wavelengths and devised a protocol that provides results equivalent to differential absorption coefficient measurements [Fig. 7(b)].

The concept of utilizing two optical wavelengths amplitude-modulated with two square waves operating at the same frequency but in opposite phase, was first introduced for aqueous glucose measurements based on absorption spectroscopy measurements [31]. This technique enables one to perform differential absorption

coefficient measurements, leading to two benefits: i) suppression of the background provided by water solvent and ii) emphasis on the effects of the small difference in the optical absorption coefficients. To further develop this method and overcome the limitation inherent to the purely optical technique, a similar excitation sequence was combined with PA detection scheme [46]. However, despite promising results obtained *in vivo*, the proposed method still depended on several parameters other than the absorption coefficient.

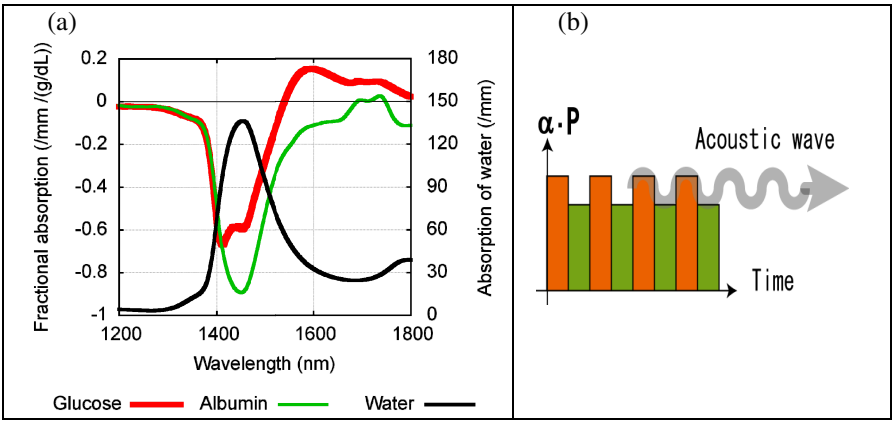

Fig. 7. (a) Spectrophotometer-based optical characteristic measurements of water, and glucose and albumin from aqueous phase and (b) a schematic view of the differential dual wavelength excitation sequence

2.2.2 Measurement Procedure

Figure 8 shows a schematic view of the experimental setup used to perform OPBS measurements with one pair of optical wavelengths. The system is similar to the one depicted in Fig. 2, except that this time, the frequency generator (FG) drives two laser diodes (LDs) drivers at the opposite phase. By using the two channels of the FG, amplitude levels on the two channels can be adjusted independently as well.

The use of two optical wavelengths according to the aforementioned scheme generates acoustic waves S in the medium, where the pressure S can be described as [33]

$$S \propto \frac{\beta v \alpha_1}{C_p} P_1 - \frac{\beta v \alpha_2}{C_p} P_2 = \frac{\beta v}{C_p} (\alpha_1 P_1 - \alpha_2 P_2) \tag{3}$$

with S is the acoustic signal (linearly proportional to the output voltage from the transducer), β the thermal expansion coefficient, v the acoustic velocity, C_p the heat capacity, α the optical absorption coefficient, and P the optical power; the

subscript 1 or 2 relates parameters to optical wavelength 1 or 2. The acoustic wave generation still involves several parameters. In order to suppress the influence of all parameters except the optical absorption, we then proposed the OPBS protocol, which can be summarized in one sentence as follows: the change in optical absorptions at the two wavelengths coming from the change in the concentration is compensated by adjusting the optical output power of the LDs.

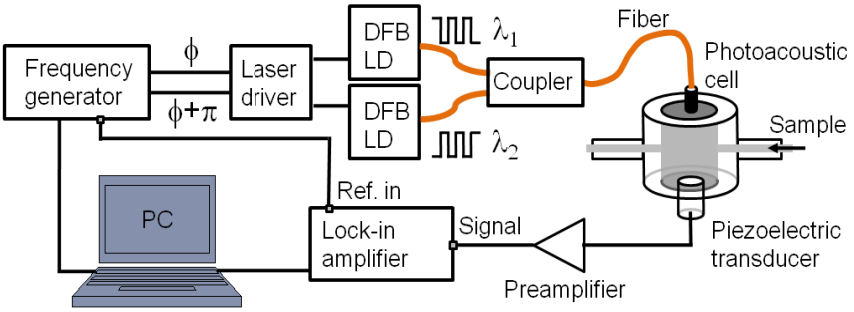

Fig. 8. Schematic view of the experimental setup used to perform OPBS measurements with one pair of optical wavelengths

Since the OPBS method is a relative measurement, and therefore requires a reference, the measurement sequence can be decomposed into two steps (Fig. 9). With a sample solution at a known concentration, a first amplitude/phase signal is captured by scanning over the driving voltage (DV) of the two LDs [Fig. 9(a)]. On the left side of the graph, the contribution from wavelength 1 (λ_1, orange) is dominant, and leads to a high amplitude signal level as well as a phase consistent with λ_1. On the opposite side of the graph, the contribution from wavelength 2 (λ_2, green) is dominant, leading to a high amplitude signal level and a phase consistent with λ_2. When comparing the signals on the two sides, the amplitude exhibits similar levels, while the phase shows a 180° phase difference because of the phase difference between λ_1 and λ_2. In between, the amplitude exhibits a minimum and the phase an inflexion point. At this particular condition, the DVs of the two LDs minimize the parameter $\Delta(\alpha P)$, which result in almost the non-generation of acoustic wave. This point then serves as a reference 0 at the known concentration level [X g/dL glucose concentration in Fig. 9(a)].

When the glucose concentration is changed (from X to X+δX g/dL), another scan of the LDs' DVs reveals a pattern similar in shape to the reference one but overall shifted by a certain amount [Fig. 9(b)]. This shift comes from the fact that changing the concentration of the dilute compound affects the two optical absorption coefficients α_1 and α_2. Therefore, the combination of LDs' output power that minimizes the parameter $\Delta(\alpha P)$, is no more at the 0-reference level, but shifted by a quantity proportional to the change of α_1 and α_2.

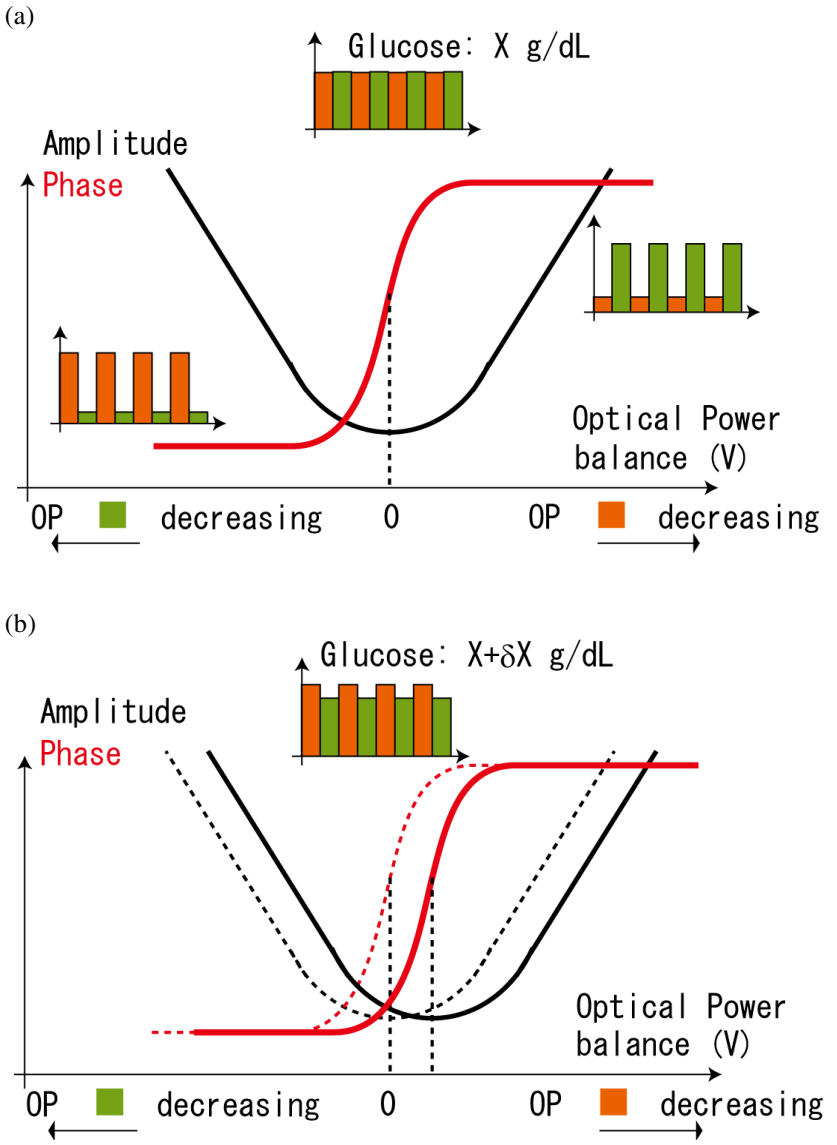

Fig. 9. Two-step process of the OPBS procedure: (a) measurement of a standard sample solution at known concentration, followed by (b) the measurement of sample solution at unknown concentration level

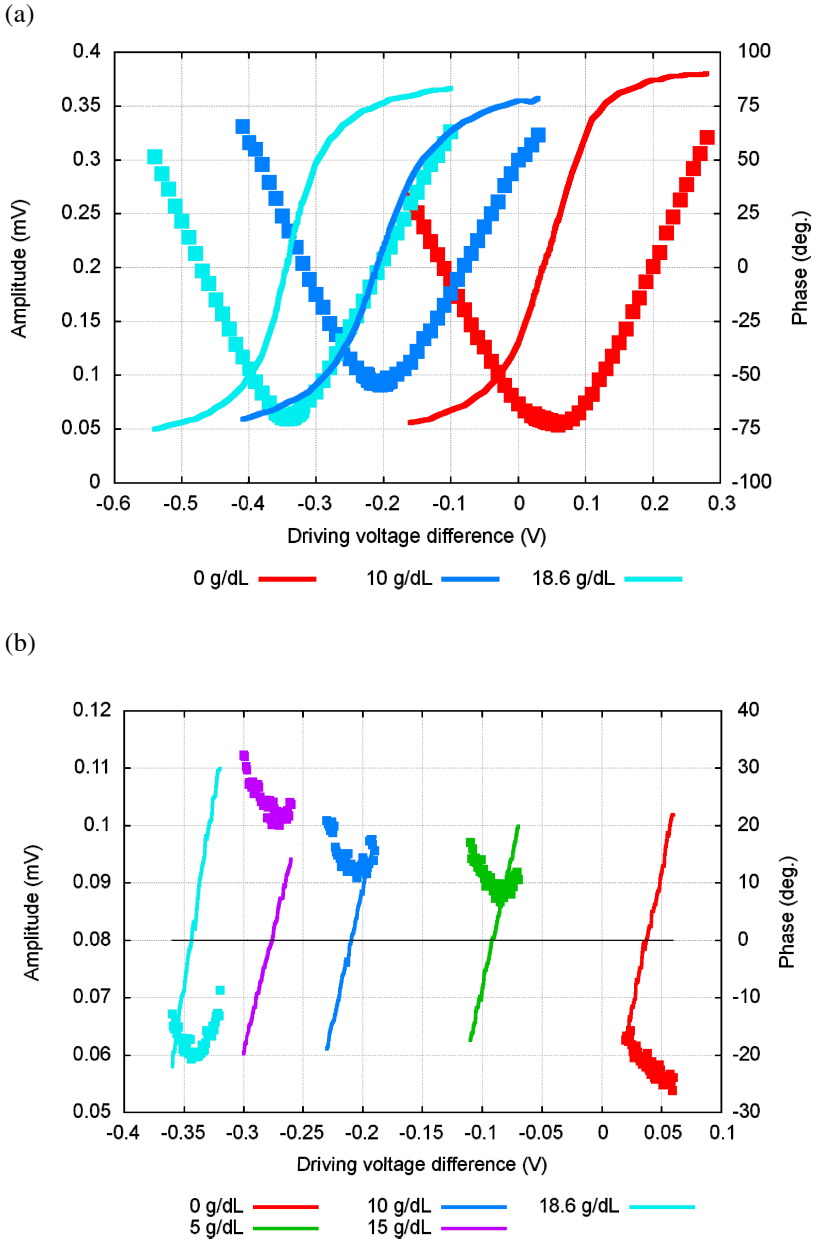

Fig. 10. Amplitude (dots) and phase (lines) signals from OPBS measurements of various aqueous solutions of glucose; (a) wide DV balance scan for three concentrations and (b) close-views around the phase inflexion points

According to this experimental procedure, only and exclusively the term between brackets in Eq. (3) plays a role. Unlike the process described elsewhere, where the signal level at one fixed DV balance was used [46], the effect of all the other parameters such as heat capacity and thermal expansion doesn't interfere with OPBS measurements.

The proposed approach then exhibits the advantages of optical absorption-based protocols (versatility from the optical wavelengths choice) and differential measurement (suppression of background), without the limitation inherent to purely optical measurement (scattering, light path length extended for improved accuracy, but limited by penetration depth).

2.2.3 Results for the 1382- and 1610-nm Combination

At the present time, only two LDs are available, leading to only one wavelength combination. Figure 10 shows the experimental raw results for glucose aqueous solution with concentrations ranging from 0 to 18.6 g/dL. The DV difference on the x-axis is arbitrarily defined as DV_2-DV_1. From a practical point of view, an LD's DV cannot exceed a certain threshold in order to operate within the linear range. For instance, once DV_1 has reached its maximum value, DV_2 is decreased instead of further increasing DV_1. Furthermore, a phase offset of 90 deg. was set on the lock-in amplifier so that the phase varied from -90 to 90 deg. with an inflexion point at 0 deg. Figure 10(a) exhibits an overall shape consistent with Fig. 9, and Fig. 10(b) only focuses around the inflexion point of the phase at the various concentration.

The phase exhibits linear behavior locally and enables easy and fast determination of the DV balance that corresponds to a 0-phase. On the contrary, the amplitude shows a minimum point, but the higher noise level makes the data processing difficult. Despite a remaining shift between the 0-phase and amplitude minimum point (unexplained at the present time), we chose to use exclusively the 0-phase point for the next results. Figure 11 then gathers all the $\Delta(DV)|_{phase=0}$ for each glucose solution, as well as equivalent results for albumin aqueous solutions at various concentration levels, all normalized to water.

The dependence is more pronounced for glucose. Despite our changing only the concentration of one compound at a time, the results are not perfectly aligned and the error-bars remain large. In terms of sensor response, we could then evaluate the slope between -20 and -27 mV/g/dL for glucose, and between -6 and -12 mV/g/dL for albumin (solid lines in Fig. 11 for both compounds). However, this behavior confirms the tendency expected from Fig. 7(a) with the two optical wavelengths used here (response to glucose about 2.41 times higher than the response to albumin). Furthermore, both uncertainties resulted from the same experimental issue about controlling the temperature during the measurement. The OPBS approach is also sensitive to temperature due to the temperature dependence of the absorption coefficient [47]. The detection cell containing the liquid

sample was then fully immersed in large quantity of water in order to drastically increase the thermal inertia. The fast fluctuation of temperature could be suppressed, but a gradual and constant drift of the temperature could be measured during the daytime. The error-bars were calculated from temperature measurement of the water outside the cell and its potential impact on the PA signals inside the cell. Related with the experimental sequence (increasing the concentration levels during the day), the measurement errors increase with the concentration levels. Another drawback of the OPBS approach, which is not taken into account in the error-bar calculation, arises from the fact that it utilizes the PA amplitude levels. Therefore, any fluctuation of the output of the LD itself will affect the OPBS signal and can then be misinterpreted as a change in glucose compound concentration.

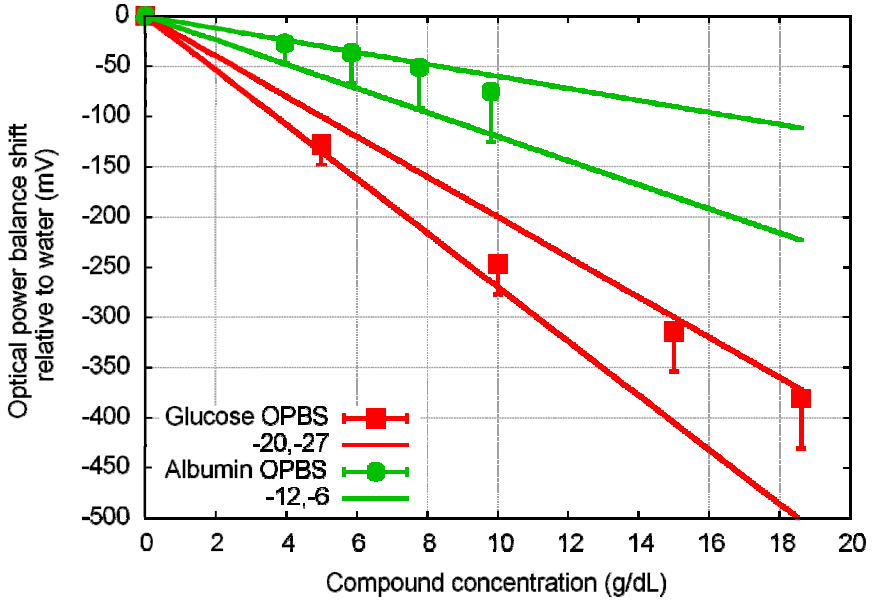

Fig. 11. OPBS results relative to water for various concentration levels of glucose and albumin aqueous solutions

3 FS+OPBS Combination

3.1 Comparison of the Two Approaches

The PA technique mixes optical excitation and acoustic detection. The two methods previously developed rely on the two aspects separately: the FS method depends exclusively on the acoustic part and characterizes the propagation

medium through acoustic velocity measurements, while the OPBS method senses any change of the absorption coefficients at the two optical wavelengths. Therefore, the two methods exhibit very different characteristics. Table 1 shows the FS and OPBS dependence versus three of the main parameters regarding the issue of a non-invasive blood glucose sensor: glucose and albumin concentrations, and temperature.

Table 1. Comparison of the FS and OPBS dependence versus glucose concentration, albumin concentration, and sample temperature

	FS method	***OPBS method***
Glucose	0.19 %/g/dL	-20 ~ -27 mV/g/dL
Albumin	0.15 %/g/dL	-6 ~ -12 mV/g/dL
Temperature	0.16 %/degC	25 mV/degC

With the FS method, the sensitivity to the three parameters is about the same order of magnitude and no optimization is possible, since the results directly and exclusively depend on the acoustic velocity dependence versus these three parameters. However, the FS relies on a frequency measurement, which also provides high sensitivity and accuracy.

The combination of the two protocols may then be the best solution by bringing together the high sensitivity of the FS approach and the high selectivity to glucose compound of the OPBS method.

Regarding the OPBS protocol, the dependence versus the three parameters varies consequently. Furthermore, the method's response can be tuned by changing the optical wavelengths so that further optimization towards high sensitivity to one specific compound is possible. As a result, OPBS enables compound-selective measurements. However, the method relies on amplitude-based measurements, which limits its sensitivity due to noise and instability.

3.2 Creation of Linear System

From the results in Fig. 11, one can see that temperature fluctuation during the measurements makes quite difficult to estimate precisely the sensor response to the two compounds. However, the experimental protocol requires an evaluation of the frequency shift (FS) prior to any OPBS measurements. For the two FS and OPBS measurements performed within minutes from each other (Fig. 12), we can then reasonably assume a constant temperature and compound concentration.

From the four experimental sets of FS+OPBS data points, we then built a system of eight equations that involve twelve parameters for each set of data: the OPBS and FS slope responses to glucose (or albumin) and temperature and the four temperature differences and compound concentration differences.

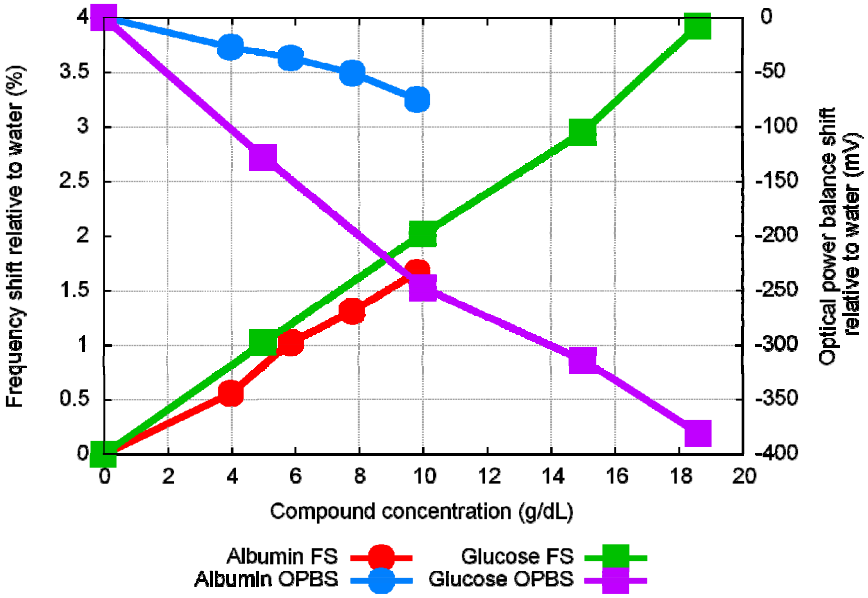

Fig. 12. Raw responses for the FS and OPBS methods versus albumin and glucose concentrations

Among these twelve parameters, the compound concentration differences are known (preparation of the sample solution), as are the FS slope response to the solute and the FS and OPBS slope responses to temperature. In this particular case, we then have an over-determined system with eight equations and five unknown parameters, which can be solved according to one of several mathematical tools already available. We then obtain the two sets of results: the temperatures profile (Fig. 13) and the OPBS response slope (Fig. 14).

Figure 13 shows the estimated variations of the temperature versus the experiment number. The experiments were performed sequentially with increasing concentrations, so that the low experiment numbers correspond to low-concentration samples. We obtained an increase of temperature as the experiment number increases, within a range that is compatible with our first observations except for the last data point with glucose. Figure 14 shows the experimental results superimposed with the fitting curves, whose slopes are -24 and -10 mV/g/dL for glucose and albumin, respectively. Because we took into account FS and OPBS data

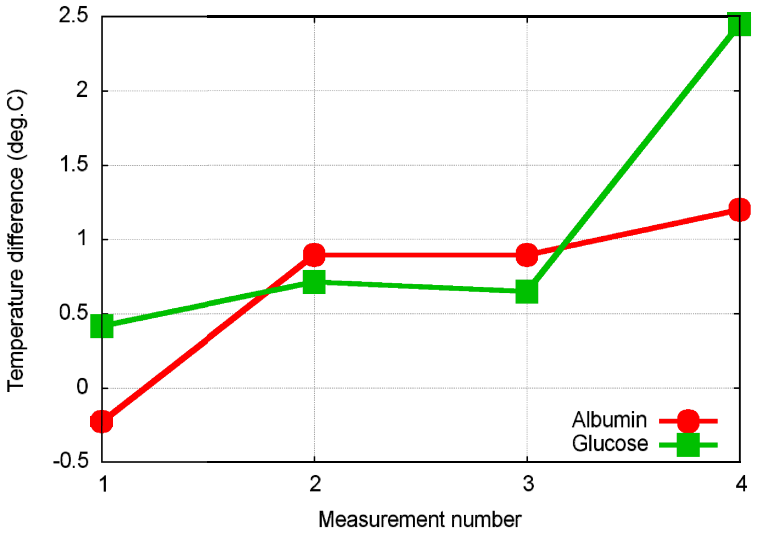

Fig. 13. Estimated temperature profile during the experiments performed with glucose and albumin solutions

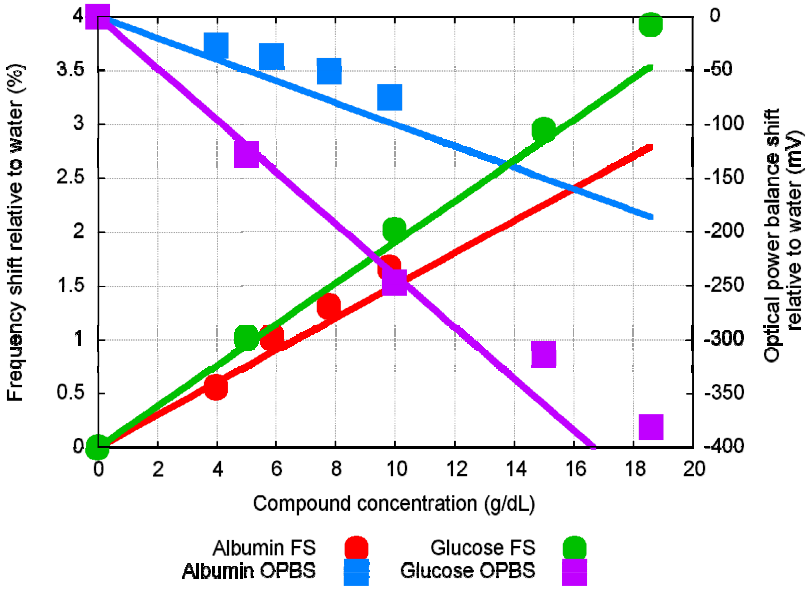

Fig. 14. Sensor responses for the FS and OPBS methods versus albumin and glucose (dots) with corresponding slopes obtained from solving the combined problem

simultaneously, the results do not really look intuitive when considering exclusively the OPBS method. However, we can compare those results with the spectroscopic data from Fig. 7(a): if we consider the slope ratio defined as the response to glucose divided by the response to albumin, we get 2.40 compared to the 2.41 value expected from optical absorbance measurement by calculating (relative absorption(λ_1) - relative absorption(λ_2))$_{Glucose}$ / (relative absorption(λ_1) - relative absorption(λ_2))$_{Albumin}$).

3.3 Solution to Multi-parameter Problem

However, the previously described process is just a demonstration of the concept. In real experiments, glucose is the target molecule, and the issue of the increasing the number of linearly independent equations will then be resolved by using several optical wavelengths combinations in parallel.

Similarly to the pulse oximetry protocol [48,49], we can extend the approach to N optical wavelengths to specifically measure the glucose concentration from an environment featuring several parameters varying simultaneously. With N LD operating at N different optical wavelengths, it is then possible to obtain N(N-1)/2 combinations of two optical wavelengths for OPBS measurements. Since the sensitivity to compounds varies as a function of the optical wavelengths, we can now get N(N-1)/2 equations versus the identified M unknown parameters. However, all these equations are not independent, and only (N-1) can be used to solve the problem. With the FS measurement at one optical wavelength (any wavelength can be used for this measurement), we then have a system including N independent equations and M unknown parameters. The M unknown parameters, already including the glucose and albumin concentrations, as well as temperature, can also be extended to take into account other compounds or parameters that may vary and influence the sensor responses in a detectable way. After fixing M, N can be adjusted freely with $N \geq M$ in order to get a complete system. Nevertheless, while the measurement accuracy increases proportionally to N, the sensor response time and cost will also increase as a consequence, so that a compromise may be found depending on the requirements in terms of accuracy and response time.

4 Conclusions

We proposed a novel concept of non-invasive glucose concentration measurement based on the CW-PA principle. One the one hand, the FS method provides a measurement equivalent to acoustic velocity monitoring, thus taking into account several different parameters (such glucose and albumin concentrations, and temperature). Despite having no selectivity to one compound in particular, it also enables to correct the frequency shift induced by the change of the acoustic properties of the medium and therefore provides a system that works with equal efficiency whatever the cavity size. On the other hand, the OPBS method is equivalent to a differential relative absorption coefficient measurement at the two wavelengths used and thus opens the door to optimization/customization based on

the excitation wavelength choice. Finally, by combining the FS and OPBS protocols, which rely on different properties of the PA technique, we designed a tunable system that can potentially solve complex problems involving many parameters. Moreover, the PA detection scheme provides robustness against scattering properties of tissues. This method then appears suitable for *in vivo* BGL monitoring, where several compounds can bias the measurement of glucose, the primary target molecule, and the scattering of tissue cannot be neglected.

Furthermore, the properties demonstrated by the FS+OPBS method can potentially fulfill the requirements of numerous applications, where the noninvasive term may be replaced by other terms such as inline monitoring or contactless evaluation. In all cases, the basic idea remains the same: while the sample solution is inside a close container that does not allow direct access or contact with the liquid, FS+OPBS may provide a viable alternative for remote characterization by adapting the wavelengths to the solute/solvent considered, especially when scattering from the sample solution cannot be neglected.

References

[1] Wild, S., Roglic, G., Green, A., Sicree, R., King, H.: Global prevalence of diabetes, Estimates for the year 2000 and projections for 2030. Diabetes Care 27(5), 1047–1053 (2004)

[2] World Health Organization (2011), http://www.who.int/mediacentre/facsheets/fs312/en/

[3] Roglic, G., Unwin, N., Bennett, P.H., Mathers, C., Tuomilehto, J., Nag, S., Connolly, V., King, H.: The burden of mortality attributable to diabetes. Diabetes Care 28(9), 2130–2135 (2005)

[4] World Health Organization, International Diabetes Federation (2004), http://www.who.int/diabetes/publications/diabetes_booklet/en/index.html

[5] The Expert Committee on the Diagnosis and Classification if Diabetes Mellitus. Follow-up report on the diagnosis of diabetes mellitus, Diabetes Care 26(11), 3160–3167 (2003)

[6] American Diabetes Association. Standards of medical care in diabetes, Diabetes Care 28 (supp.1), s4–s36 (2005)

[7] Consensus Development Panel. Consensus statement on self-monitoring of blood glucose. Diabetes Care 10, 95–99 (1987)

[8] Kirk, J.K., Stegner, J.: Self-monitoring of blood glucose: practical aspects. J. Diabetes Sci. Technol. 4(2), 435–439 (2010)

[9] Rubin, A.L.: Diabetes for dummies, 3rd edn. Wiley Publishing, Inc. (2008)

[10] Thorsell, A., Gordon, M., Jovanovic, L.: Continuous glucose monitoring: a stepping stone in the journey towards a cure for diabetes. J. Maternal-Fetal and Neonatal Medicine 15(1), 15–25 (2004)

[11] Sachedina, N., Pickup, J.C.: Performance assessment of the Medtronic-MiniMed continuous glucose monitoring system and its use for measurement of glycaemic control in type 1 diabetic subjects. Diabetic Medicine 20(12), 1012–1015 (2003)

[12] Ginsberg, B.H.: An overview of minimally invasive technologies. Clin. Chem. 38(9), 1596–1600 (1992)

[13] Kost, J., Mitragotri, S., Gabbay, R.A., Pishko, M., Langer, R.: Transdermal monitoring of glucose and other analytes using ultrasound. Nature Medicine 6(3), 347–350 (2000)
[14] Chuang, H., Taylor, E., Davison, W.: Clinical evaluation of a continuous minimally invasive glucose flux sensor placed over ultrasonically permeated skin. Diabetes Techn. & Therapeutics 6(1), 21–30 (2004)
[15] Erdman, C.P., Goldman, S.M., Lynn, P.J., Ward, M.C.: A minimally inva-sive device for continuous glucose monitoring in infants. J. Med. Devices 2(2), 027518 (2008)
[16] Hanaire, H.: Continuous glucose monitoring and external insulin pump: towards a subcutaneous closed loop. Diabetes & Metabolism 32(5), 534–538 (2006)
[17] Steil, G.M., Rebrin, K., Goode Jr., P.V., Mastrototaro, J.J., Purvis, R.E., Van Antwerp, W.P., Shin, J.J., Talbot, C.D.: Closed loop system for controlling insulin infusion. US Patent US006558351B1 (2003)
[18] Chee, F., Fernando, T.: Closed-loop control of blood glucose. LNCIS, vol. 368. Springer Ed. (2007) ISBN 3-540-74030-9
[19] Heo, Y.J., Shibata, H., Okitsu, T., Kawanishi, T., Takeuchi, S.: Long-term in vivo glucose monitoring using fluorescent hydrogel fibers. PNAS 1104954108 (2011)
[20] Voskerician, G., Anderson, J.: Foreign body reaction. Wiley Encyclopedia of Biomedical Engineering (2006) ISBN 9780471740360
[21] Ma, K., Yuen, J.M., Shah, N.C., Walsh Jr., J.T., Glucksberg, M.R., Van Duyne, R.P.: In vivo, transcutaneous glucose sensing using surface-enhanced spatially offset Raman spectroscopy: multiple rats, improved hypoglycemic accuracy, low in-cident power, and continuous monitoring for greater than 17 days. Anal. Chem. 83(23), 9146–9152 (2011)
[22] Wagner, J., Malchoff, C., Abbott, G.: Invasiveness as a barrier to self-monitoring of blood glucose in diabetes. Diabetes Techn. & Therapeutics 7(4), 612–619 (2005)
[23] Pickup, J.C., Hussain, F., Evans, N.D., Sachedina, N.: In vivo glucose monitoring: the clinical reality and the promise. Biosens. and Bioelect. 20, 1897–1902 (2005)
[24] Heinemann, L., Schmelzeisen-Redeker, G.: Non-invasive continuous glucose monitoring in Type I diabetic patients with optical glucose sensors. Diabetologia 41, 848–854 (1998)
[25] Khalil, O.S.: Non-invasive glucose measurement technologies: an update from 1999 to the dawn of the new millennium. Diabetes Techn. & Therapeutics 6(5), 660–697 (2004)
[26] Tura, A., Maran, A., Pacini, G.: Non-invasive glucose monitoring: assessment of technologies and devices according to quantitative criteria. Diabetes Research and Clinical Practice 77, 16–40 (2007)
[27] MacKenzie, H.A., Ashton, H.S., Shen, Y.C., Lindberg, J., Rae, P., Quan, K.M., Spiers, S.: Blood glucose measurements by photoacoustics. Biomedical Opt. Biomedical Opt. Spectroscopy and Diagnostics/Therapeutic Laser App. 22, 156–159 (1998)
[28] Zhao, Z.: Pulse photoacoustic techniques and glucose determination in human blood and tissue. Oulu University Press, Oulu (2002) ISBN 951-42-6689-7
[29] Weiss, R., Yegorchikov, Y., Shusterman, A., Raz, I.: Noninvasive continuous glucose monitoring using photoacoustic technology- Results from the first 62 subjects. Diabetes Technology and Therapeutics 9(1), 68–74 (2007)
[30] Patel, C.K.N., Tam, A.C.: Pulsed optoacoustic spectroscopy of condensed matter. Rev. of Modern Physics 53(3), 517–553 (1981)
[31] Spanner, G., Niessner, R.: New concept for the non-invasive determination of physiological glucose concentrations using modulated laser diodes. Fresenius J. Anal. Chem. 354, 306–310 (1996)

[32] Tam, A.C.: Applications of photoacoustic sensing techniques. Reviews of Modern Physics 58(2), 381–431 (1986)
[33] Atalar, A.: Photoacoustic effect as a liquid absorbance detector. Applied Optics 19(18), 3204–3210 (1980)
[34] Miklos, A., Hess, P., Bozoki, Z.: Application of acoustic resonators in photoacoustic trace gas analysis and metrology. Review of Scientific Instruments 72(4), 1937–1955 (2001)
[35] Hippler, M., Mohr, C., Keen, K.A., McNaghten, E.D.: Cavity-enhanced resonant photoacoustic spectroscopy with optical feedback cw diode lasers: A novel technique for ultratrace gas analysis and high-resolution spectroscopy. J. Chem. Phys. 133, 044308 (2010)
[36] Havey, D.K., Bueno, P.A., Gillis, K.A., Hodges, J.T., Mulholland, G.W., van Zee, R.D., Zachariah, M.R.: Photoacoustic spectrometer with a calculable cell constant for measurements of gases and aerosols. Anal. Chem. 82(19), 7935–7942 (2010)
[37] Camou, S., Ueno, Y., Tamechika, E.: Towards non-invasive and continuous blood sugar sensor: detection of aqueous glucose based on CW-photoacoustic proto-col. In: Proceeding of IEEE Sensors 2010, Hawaii, USA (2010)
[38] Camou, S., Haga, T., Tajima, T., Tamechika, E.: Detection of aqueous glucose based on a cavity size- and optical-wavelength-independent continuous-wave photoacoustic technique. Anal. Chem. 84(11), 4718–4724 (2012)
[39] Hao, L.-Y., Ren, Z., Shi, Q., Wu, J.-L., Zheng, Y., Zheng, J.-J., Zhu, Q.-S.: A new cylindrical photoacoustic cell with improved performance. Review of Scientific Instruments 73(2), 404–410 (2001)
[40] Shen, Y., Spiers, S., MacKenzie, H.A.: Time resolved aspects of pulsed photoacoustic spectroscopy. Analytical Sciences 17, Special Issue, 221–222 (2001)
[41] Zips, A., Faust, U.: Determination of biomass by ultrasonic measurements. Appl. Environ. Microbiol. 55(7), 1801–1807 (1989)
[42] Hall, J.W., Pollard, A.: Near-infrared spectrophotometry: a new dimension in clinical chemistry. Clin. Chem. 38(9), 1623–1631 (1992)
[43] Chung, H., Arnold, M.A., Rhiel, M., Murhammer, D.W.: Simultaneous measurements of glucose, glutamine, ammonia, lactate, and glutamate in aqueous solutions by near-infrared spectroscopy. Applied Spectroscopy 50(2), 270–276 (1996)
[44] Ward, K.J., Haaland, D.M., Robinson, M.R., Eaton, R.P.: Post-prandial blood glucose determination by quantitative mid-infrared spectroscopy. Applied Spectroscopy 46(6), 959–965 (1992)
[45] Heise, H.M., Marbach, R., Koschinsky, T., Gries, F.A.: Multicomponent assay for blood substrates in human plasma by mid-infrared spectroscopy and its evaluation for clinical analysis. Applied Spectroscopy 48(1), 85–95 (1994)
[46] Spanner, G., Niessner, R.: Noninvasive determination of blood constituents using an array of modulated laser diodes and a photoacoustic sensor head. Fresenius J. Anal. Chem. 355, 327–328 (1996)
[47] Jensen, P.S., Bak, J., Andersson-Engels, S.: Influence of temperature on water and aqueous glucose absorption spectra in the near- and mid-infrared regions at physiologically relevant temperatures. Applied Spectroscopy 57(1), 28–36 (2003)
[48] Allen, K.: Principles and limitations of pulse oximetry in patient monitoring. Nurs. Times 12-18, 100(41), 34–37 (2004)
[49] Mendelson, Y.: Pulse oximetry: theory and applications for non-invasive monitoring. Clin. Chem. 38(9), 1601–1607 (1992)